

*Rhythms in Fishes -
A Tribute To Milt Stetson*

SYMPOSIUM PROCEEDINGS

Benny Ron

Don MacKinlay

International Congress on the Biology of Fish
Tropical Hotel Resort, Manaus Brazil, August 1-5, 2004

Copyright © 2004
Physiology Section,
American Fisheries Society
All rights reserved

International Standard Book Number (ISBN) 1-894337-43-3

Notice

This publication is made up of a combination of extended abstracts and full papers, submitted by the authors without peer review. The formatting has been edited but the content is the responsibility of the authors. The papers in this volume should not be cited as primary literature. The Physiology Section of the American Fisheries Society offers this compilation of papers in the interests of information exchange only, and makes no claim as to the validity of the conclusions or recommendations presented in the papers.

For copies of these Symposium Proceedings, or the other 20 Proceedings in the Congress series, contact:

Don MacKinlay, SEP DFO, 401 Burrard St
Vancouver BC V6C 3S4 Canada
Phone: 604-666-3520 Fax 604-666-0417
E-mail: mackinlayd@pac.dfo-mpo.gc.ca

Website: www.fishbiologycongress.org

RHYTHMS IN FISHES SYMPOSIUM

A TRIBUTE TO MILTON STETSON

1943 - 2002

Professor Milton H. Stetson died on June 27, 2002, while working on a sabbatical leave at the Hawaii Institute of Marine Biology of the University of Hawaii. Born in 1943 in Springfield, MA, to Milton and Evelyn Stetson, he later moved to Windsor, CT. After high school, he continued his education at Central Connecticut University in New Britain. He received his Master and Doctorate degrees from the University of Washington in Seattle.

Dr. Stetson served as a Postdoctoral Fellow under Michael Menaker at the University of Texas at Austin. Milt Stetson came to the University of Delaware in 1973, where he served as Professor, and Associate Director of Graduate Programs and Research in the School of Life and Health Sciences, and Director of the School of Life and Health Sciences. In 1998, he became an Affiliate Professor at the Hawaii Institute of Marine Biology, a marine station of the University of Hawaii, situated on Coconut Island, Kaneohe Bay, Oahu, Hawaii.

While serving at the University of Delaware, Professor Stetson authored, co-authored, or edited nearly 200 scientific publications in various research areas, such as vertebrate reproduction, neuroendocrinology, comparative endocrinology, circadian physiology, pineal physiology and vertebrate photoperiodism. In addition, he trained 13 graduate students, 10 of whom received the degree of PhD. Professor Stetson served on many committees, refereed articles for several scientific journals, and refereed grants for NSF, NIH, USDA and EPA.

Always an avid fisherman, Milt Stetson knew how enjoyed also golf, gardening, crossword puzzles, bowling, and reading. We all remember Milt by all for his enormous generosity and his outrageous sense of humor.

Symposium Organizers:

Benny Ron, National Centre for Mariculture, Israel
Don MacKinlay, Fisheries and Oceans Canada

CONGRESS ACKNOWLEDGEMENTS

This volume is part of the Proceedings of the 6th International Congress on the Biology of Fish, held in Manaus, Brazil in August, 2004. Ten years have passed since the first meeting in this series was held in Vancouver, BC, Canada. Subsequent meetings were in San Francisco, California; Baltimore, Maryland; Aberdeen, Scotland; and again in Vancouver, Canada. From those meetings, colleagues from over 30 countries have contributed more than 2,500 papers to the Proceedings of over 80 Congress Symposia, all available for free viewing on the internet.

We would like to extend our sincere thanks to the many people who helped us organize the facilities and program for this 6th Congress.

The local arrangements team worked very hard to make this Congress a success. The leaders of those efforts were Vera Almeida Val, Adriana Chippari-Gomes, Nivia Pires Lopes and Maria de Nazare Paula Silva (Local Arrangements); Marcelo Perlingeiro (Executive Secretary) and Maria Angelica Laredo (Fund Raising). The enormous contribution of time and effort that was required has led to an unforgettable experience for the participants, thanks to the imagination, determination and dedication of this team.

Many sponsors helped ensure the success of the meeting through both monetary and in-kind contributions, including: Fundação Djalma Batista, Honda, Merse, Cometais, Turkys Aquarium, Banco da Amazônia, Banco do Brasil, FUCAPI, SEBRAE/AM, IDAM/SEPROR, FAPEAM, SECT-AM, SUFRAMA, PETROBRÁS, CAPES, FINEP, CNPq, the Physiology Section of the American Fisheries Society, UFAM - Federal University of Amazonas, Fisheries and Oceans Canada and INPA - National Institute for Research in the Amazon.

Travel arrangements were ably handled by Atlantic Corporate Travel (special thanks to Maria Espinosa) and Orcal Planet, and the venue for the meeting was the spectacular Tropical Hotel Conference Center in Manaus.

The Student Travel Award Committee of the Physiology Section of the American Fisheries Society, led by Michael Redding, evaluated 65 applications from 15 countries and awarded 40 Travel Grants, after an ambitious and trying fund-raising effort. Special thanks must go the US Department of Agriculture, the US Geological Survey, US National Science Foundation and the World

Fisheries Congress for providing funds. In addition, the American Fisheries Society contributed books to be used as prizes for the best student papers.

The editorial team compiled the short abstracts into an abstract book and formatted and compiled the papers for the Symposium Proceedings. Thanks to Karin Howard, Christie MacKinlay, Anne Martin, Callan MacKinlay and Marcelo Perlingeiro.

In particular, we would like to extend a sincere 'thank you' to the organizers of the individual scientific Symposia and their many contributors who took the time to prepare a written submission for these proceedings. Their efforts are very much appreciated. We hope that their participation will result in new insights, new collaborations and new lines of research, leading to new papers to be presented at the 2006 Congress in St. John's, Newfoundland.

Congress Chairs:

Adalberto Luis Val
National Institute for Research
in the Amazon, INPA,
Manaus, Brazil

Don MacKinlay
Fisheries & Oceans Canada
Vancouver, Canada

TABLE OF CONTENTS

Importance of Lunar-cues in Synchrony of Reproductive Cycle in Reef Fishes <i>Akihiro Takemura</i>	1
Influence of lunar cycles on locomotor activity rhythms in tench (<i>Tinca tinca</i>) exposed to natural moonlight. <i>García-Vizcaíno, E.M.; Herrero, M.J; Madrid, J.A. & Sánchez-Vázquez, F.J.</i>	5
Influence of light on nocturnal plasma melatonin and locomotor activity in tench (<i>Tinca tinca</i>) <i>Vera, L.M.; López-Olmeda, J.F.; Bayarri, M.J.; Madrid, J.A.; Sánchez-Vázquez, F.J.</i>	9
The influence of the pineal organ and melatonin on the reproductive system and of light intensity and wavelength on melatonin in the gilthead sea bream (<i>Sparus aurata</i>) <i>Alon Naor, Eran Hadas and Benny Ron</i>	13
Melatonin effects on metabolism and behavioural rhythms in goldfish <i>López-Olmeda, J.F.; Bayarri, M.J.; Rol de Lama, M.A.; Madrid, J.A.; Sánchez-Vázquez, F.J</i>	19
Estimation of plasma melatonin fluctuation by cannulation <i>Yoshiaki Nikaido</i>	23
Ontogeny of the pineal melatonin-generating system in the gilthead seabream (<i>Sparus aurata</i>) <i>Galit Lisaey, Yoav Gothilf and Benny Ron</i>	27
Differential regulation of two arylalkylamine-n-acetyltransferase in the gilthead seabream (<i>Sparus aurata</i>) <i>Nitzan Segev, Bina Zilberman-Peled, Yoav Gothilf and Benny Ron</i>	31
Chronology and food consumption of white croaker (<i>Micropogonias furnieri</i>), an estuarine dependent fish. <i>G.M. Figueiredo & J.P. Vieira</i>	39

Self-feeding rhythms and macronutrients self-selection in Senegal sole
(*Solea senegalensis*)
*Boluda-Navarro, D.; Luz, R. K.; Rubio, V. C.; Sánchez-Vázquez,
F. J.; Fernández-Pasquier, V.; Madrid, J. A*43

**IMPORTANCE OF LUNAR-CUES
IN SYNCHRONY OF REPRODUCTIVE CYCLE
IN REEF FISHES**

A. Takemura

Sesoko Station, Tropical Biosphere Research Center, University of the Ryukyus,
3422 Sesoko, Motobu, Okinawa 905-0227, Japan
+81-980-47-6215/+81-980-47-4919
tilapia@lab.u-ryukyu.ac.jp

M.S. Rahman, Y.J. Park, Y. Nikaido, S.S. Endang and K. Takano
Sesoko Station, Tropical Biosphere Research Center, University of the Ryukyus,
3422 Sesoko, Motobu, Okinawa 905-0227, Japan

EXTENDED ABSTRACT ONLY - DO NOT CITE

General views of lunar synchronization of fishes

Complex associations of the sun-earth-moon orbit causes changes in tide and moonlight intensity on the earth. It has been reported that many reef fishes utilize such rhythmic changes for synchrony of recruitment, development and release of gametes. At present, however, some of the evidence related to lunar-related reproductive rhythm are circumstantial or unconfirmed, because most studies have been carried out based upon field observations of spawning behavior and occurrence of spawned eggs. The lack of definitive physiological studies compounds the problems for determining the lunar synchrony of reproduction. Understanding the participation of the moon in the synchrony of biological activities in fishes is significant from the perspective of aquaculture as well as chronobiology.

There are diverse adaptive strategies to lunar synchronization among the reef fishes. They are categorized as the semi-lunar and the lunar spawning cycles, the spawnings of which roughly occur at intervals of every 2 weeks and 1 month, respectively. Certain territorial and site attached damselfishes exhibit reproductive activity with peaks near the new and/or the full moon. The

spawning periodicity relative to the time of high tide has been reported in wrasses. The advantage of these strategies (semi-lunar cycle) may be to minimize immediate egg and larval predations and facilitate their transportation to offshore locales by strongest outgoing tide.

The synchronous spawning of rabbitfishes and groupers occurs around a specific lunar phase (Rahman et al., 2003). In those cases (lunar cycle), synchronicity of spawning occurs in places without a direct effect of tidal stimulation. These examples suggest a possibility that an advantage of lunar periodicity is to be synchronized gonadal maturation and increased opportunity for encounter with appropriate partners for successful spawning.

Moonlight perception and utilization by a lunar-synchronized spawner

Very few studies have investigated lunar synchronization in teleost fishes from physiological aspects. Although lunar-related synchrony is an important rhythm, it is unclear where fish perceive cues from the moon and how fish transmit such cues as endogenous stimuli. As one of possible cues from the moon, we examined the perception and utilization of moonlight conditions by the golden rabbitfish, *Siganus guttatus*, which spawns around the first quarter moon during the reproductive season. Through experiments, we paid attention particularly to melatonin, which was measured by means of time-resolved fluoroimmunoassay (Yamada et al., 2002).

Melatonin concentrations in the blood of the fish were at low levels during daytime and then increased and peaked at the midnight (24:00). Additionally, comparison of the plasma melatonin concentrations at night revealed significant difference between the full moon (low level) and the new moon (high level). These results suggest that the plasma melatonin concentration changes not only

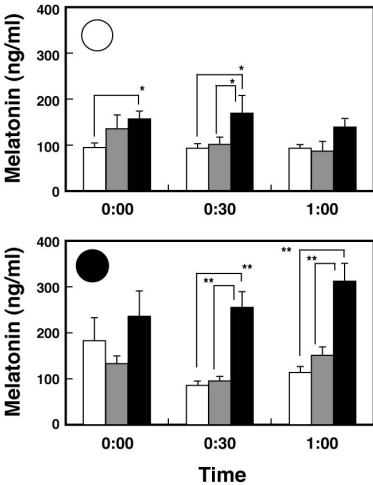


Figure 1. Changes in plasma melatonin concentration after moonlight exposure at the full moon (upper) and the new moon (lower).

with daylight intensity but also with moonlight intensity. Exposing the fish to moonlight intensity at the midnight of the new and the full moon resulted in rapid and significant decreases in the plasma melatonin concentrations to control levels (Figure 1), suggesting that the fish possibly perceives the 'brightness' of the night.

When the fish were reared under natural conditions, spawning occurred at the expected spawning dates. On the other hand, rearing the fish reared under the constant darkness and lightness of night disrupted the expected spawnings. It is possible that night conditions are related to synchronous gonadal development and spawning in the golden rabbitfish.

References

- Rahman, M.S., A. Takemura, S. Nakamura and K. Takano 2003. Rhythmic changes in testicular activity with lunar cycle in the forktail rabbitfish. *J. Fish Biol.* 62: 495-499.
- Yamada, H., H. Chiba, M. Amano, M. Iigo and M. Iwata. 2002. Rainbow trout eyed-stage embryos demonstrate melatonin rhythms under light-dark conditions as measured by a newly developed time-resolved fluoroimmunoassay. *Gen. Comp. Endocrinol.* 125: 41-46.

Acknowledgements

This study was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan (AT) and by a JSPS Research Fellowships grants for Young Scientists, Japan (MSR).

**INFLUENCE OF LUNAR CYCLES
ON LOCOMOTOR ACTIVITY RHYTHMS
IN TENCH (*Tinca tinca*)
EXPOSED TO NATURAL MOONLIGHT**

Herrero, M.J; García -Vizcaíno, E.M.;
Madrid, J.A.; Sánchez-Vázquez, F.J.
University of Murcia. Faculty of Biology. 30100 Murcia, Spain.
E-mail: mjhr@um.es

EXTENDED ABSTRACT ONLY: DO NOT CITE.

Introduction

Active movements of fish in response to external environmental variations follow cyclical patterns that with the pass of time give as a result biological rhythms endogenously driven. The periodicity of these rhythms may be ultradian, circadian and infradian. Within this last category, the circannual and the lunar rhythms are the better studied. Night-active species from shallow fresh water may be subjected to periodic changes in the brightness of moonlight and some of them synchronize their activities with the lunar day of 24.8 h or even with specific phases of the lunar month (Newmann, 1976). The experiment, kept outdoors under the direct illuminant and gravitational influence of the moon, was designed to prove the lunar effect on the locomotor activity in a fish species whose circadian daily rhythms have been reported to follow a strict nocturnal activity pattern: the tench (*Tinca tinca*). Results showed that lunar cycle affected the locomotor behaviour of tench, being a highest activity recorded under full moon.

Material and methods

The experiment was carried out in the flat roof at the top of the Biology Faculty (Murcia, Spain), with eight tench of 80 g body weight, kept in pairs in four 60-litre, fresh water aquaria, exposed to natural environmental conditions. Every

aquarium was fitted with infrared photocells (Omron, E3S-AD62, Japan) connected to a computer to continuously record fish movements. The counts made when fish interrupted the light beam of the photocells were stored every ten minutes by computer. A glass cap was disposed on the top of the aquaria and the sides were covered with black plastic film, in order to improve isolation and to prevent fish from seeing each other. Every aquarium was supplied with an individual filter and a water-heater that minimized the daily temperature variations. A constant register of the temperature was obtained through a sensor (iButton-TMEX, USA), which recorded mean temperature at 10-minute intervals. Two PVC tubes were placed in every aquarium as a shelter for the fish. Food was provided twice a week in a 2% body-weight. Aquaria were exposed to natural light conditions and photoperiod during the three lunar cycles that lasted the experiment. Stored data from photocells and temperature sensors were analysed with a chronobiology software (Temps v.1, 179 by Dr. Díez Noguera, Barcelona) and with the Excel program. The Temps program allows displaying the results in actograms which were double-plotted for a convenient visualisation.

Results

Results of the experiment developed outdoors under the direct influence of the moon on the locomotor activity of tench, showed differences under every moon phase. The locomotor activity peaked during full moon and daily activity also differed during the first and last quarter (Fig. 1), though not significant differences were found between them.

Discussion

Tench is a fish species with high sensitivity to light, developing a negative phototaxis in its locomotor activity which is synchronized to the dark phase of the photoperiod despite its length and even if it is shortened to two hours. The nocturnal behaviour of tench was kept also when differences in light intensities between the scotophase and the photophase were as minimal as LD 0.3:0 lux (Herrero et al, 2003). Such a nocturnal behaviour led us to think that the different light intensities of every lunar phase may have certain influence in the activity of tench, showing preferences for developing a more intense locomotor activity during new moon, when light intensities are at its lowest. Indeed, locomotor activity in tench was influenced by moon phases therefore, results revealed a peak of locomotor activity during the periods of full moon.

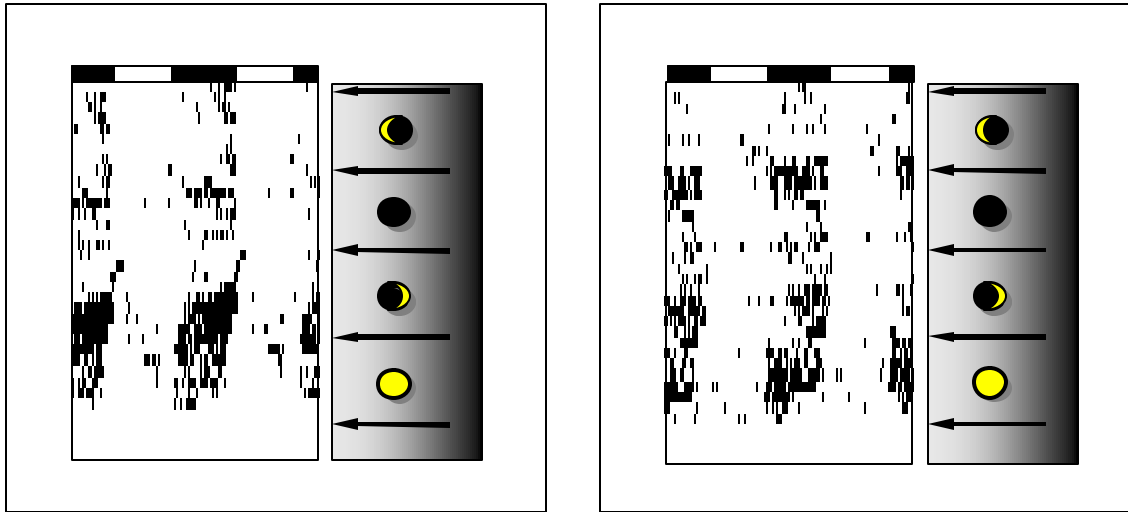


Figure 1. Actograms of locomotor activity from 2 representative tench under the influence of different lunar phases. White and black bars at the top of the graphs indicate the light and dark phases, respectively. Peak of activity was registered under full moon.

The results obtained indicated that the different light intensities might not be what synchronised lunar rhythms in tench, being discussed the importance of the illuminant influence in the lunar rhythms of tench under environmental conditions. Results about activity rhythms following the lunar cycle are consistent with those found for other fish species as *Sparus aurata* which has been reported to be under the moon influence in their feeding rhythms and indication of a maximum intake has been observed under full moon (Sánchez-Muros et al, 2003) or *Caecomastacembelus zebratus* whose proportion of feeding activity was much higher in the first and second quarters of the lunar cycle (Ochi et al, 1999).

References

- Newmann, D. Entrainment of a semilunar rhythm. In P. J. DeCoursey (Ed.), *Biological Rhythms in the Marine Environment*. Columbia: University of South Carolina Press, 1976.
- Herrero, M.J.; Madrid, J.A.; Sanchez-Vazquez, J.V. 2003. Entrainment to light of circadian activity rhythms in tench (*Tinca tinca*). *Chronobiol. Int.* 20, 6, 1001-1017.
- Sánchez-Muros, M.J.; Corchete, V.; Suárez, M.D.; Cardeneta, G.; Gómez-Millán, E.; De la Higuera, M. 2003. Effect of feeding method and protein source on *Sparus aurata* feeding patterns. *Aquaculture*, 224, 89-103.
- Ochi, H.; Sato, Y.; Yanagisawa, Y. 1999. Obligate feeding of cichlid eggs by *Caecomastacembelus zebratus* in Lake Tanganyika. *J. of Fish Biol.* 54, 450-459.

Acknowledgments

This research was supported by project AGL2001-0593-C03-01 from MCYT, awarded to FJSV.

**LIGHT PULSES INFLUENCE
ON NOCTURNAL PLASMA MELATONIN
AND LOCOMOTOR ACTIVITY
IN TENCH (*TINCA TINCA*)**

Vera, L.M.; López-Olmeda, J.F.; Bayarri, M.J.;
Madrid, J.A.; Sánchez-Vázquez, F.J.
Department of Physiology. University of Murcia. 30100 - Murcia. Spain.

EXTENDED ABSTRACT ONLY: DO NOT CITE

Introduction

Circadian rhythms in animals are entrained by external factors known as zeitgebers, with being light reported as the main zeitgeber. In teleosts, light information is transduced by the pineal organ, which is a photosensory organ containing photoreceptor cells (pinealocytes), neurons and ependymal interstitial cells. Pinealocytes are thought to be the source of melatonin synthesis. It is widely accepted that melatonin synthesis in vertebrates is regulated by the environmental light-dark cycle, peaking during the scotophase and releasing the lowest levels during the photophase. In addition, light exerts a direct action on melatonin production, suppressing the endogenous rhythm while a light pulse during the scotophase inhibits melatonin production (Falcón et al., 1987).

Recent studies have concluded that circadian activity rhythms in tench are driven by an internal circadian clock, which is strongly influenced by light. Even when light intensity was dramatically decreased during the photophase, tench remained strictly nocturnal (Herrero et al., 2003). However, the role of melatonin on light transduction under these conditions is unknown.

In the present study, we looked into the effect of light pulses of different intensities on endocrine (melatonin) and behavioural (locomotor activity) rhythms in tench.

Materials and Methods

Experiment I: effect of one hour light pulses of different intensities at MD on nocturnal melatonin production. Forty-eight tench (938 ± 22 g body weight) were used for this experiment. Fish were reared for two weeks under a 12:12 LD cycle, with an average light intensity during the daytime of $1091 \mu\text{W}/\text{cm}^2$ at the water surface. Before sampling, 32 tench were divided into four groups. Each group was exposed at mid-darkness (MD) to a one hour light pulse of different intensity (1091 , 10.5 , 5.3 or $3.3 \mu\text{W}/\text{cm}^2$). Two more groups were sampled as controls, one at mid-light (ML) and another at MD. Plasma melatonin was determined using a commercial radioimmunoassay (RIA) kit (IBL, Hamburg, Germany).

Experiment II: Seven tench (100 ± 7 g body weight) were placed in seven 60 L. glass aquaria isolated from external conditions. Locomotor activity was recorded by means of infrared photocells (OMROM E3S-AD62, Japan) placed in each aquarium. Animals were exposed to a 12:12 LD cycle during the first thirteen days and from day 14 onwards a one hour light pulse of $3.3 \mu\text{W}/\text{cm}^2$ was applied at MD.

Results

Experiment I: When a one hour light pulse was applied at MD, plasma melatonin significantly dropped to ML levels (ANOVA, $P=0.025$) at all intensities tested (1091 , 10.5 , 5.3 and $3.3 \mu\text{W}/\text{cm}^2$).

Experiment II: Locomotor activity rhythms in tench reared under LD 12:12 ($L=1091 : D=0 \mu\text{W}/\text{cm}^2$) displayed an almost strict nocturnal behaviour. However, when a $3.3 \mu\text{W}/\text{cm}^2$ light pulse was applied at MD the nocturnal locomotor activity of fish dramatically dropped. When the lights were switched off again tench resumed their activity until the photophase started.

Conclusions

The drop in plasma melatonin when a one hour light pulse is applied at MD is consistent with data obtained for other species. For instance, sea bass plasma melatonin shows great sensitivity to light, with light threshold of about 10 lux (Bayarri et al., 2002). In tench, however, melatonin showed greater sensitivity to

light, so that a light pulse intensity of $3.3 \mu\text{W}/\text{cm}^2$ (0.3 lux) dropped plasma melatonin to levels close to those obtained at ML. This suggests that melatonin rhythms may be able to transduce weak light signals such as moonlight, and synchronise lunar rhythms.

The change in the activity pattern of tench observed in exp. II, supports the previous findings, as light had a strong masking effect on tench activity rhythms, blocking their expression.

Acknowledgements

This study was supported by the Spanish Ministry of Science & Technology (MCYT) project. No. AGL2001-0593-C03-01 to FJSV and PhD fellowship to LMVA. The authors thank to the center “Vegas del Guadiana” (Badajoz) for their support.

References

- Bayarri, M.J.; Madrid, J.A.; Sánchez-Vázquez, F.J. Influence of light intensity, spectrum and orientation on sea bass plasma and ocular melatonin. *J Pineal Res* 2002, 32, 34-40.
- Falcón, J.; Guerlotti, J.; Voisin, P.; Collin, J.P. Rhythmic melatonin biosynthesis in a photoreceptive pineal organ: a study in the pike. *Neuroendocrinology* 1987, 45, 479-486.
- Herrero, M.J.; Madrid, J.A.; Sánchez-Vázquez, F.J. Entrainment to light of circadian activity rhythms in tench (*Tinca tinca*). *Chronobiol Int* 2003, 20(6), 1-17.

**THE INFLUENCE OF THE PINEAL ORGAN AND MELATONIN
ON THE REPRODUCTIVE SYSTEM
AND OF LIGHT INTENSITY
AND WAVELENGTH ON MELATONIN
IN THE GILTHEAD SEA BREAM (*SPARUS AURATA*)**

Benny Ron

National Center for Mariculture, Israel Oceanographic and Limnological
Research, P.O.B. 1212, Eilat 88112, Israel
P: 972-8-6361439
F: 972-8-6375761
ronbenny@agri.huji.ac.il

Alon Naor

National Center for Mariculture, Israel Oceanographic and Limnological
Research, P.O.B. 1212, Eilat 88112, Israel

Eran Hadas

Suf Fish Farm Ltd., North Shore, Eilat 88000, Israel

EXTENDED ABSTRACT ONLY: DO NOT CITE

Effects of full-spectrum white and monochromatic light (at various wavelengths) on pineal (*in vitro*) and plasma (*in vivo*) melatonin were investigated in the gilthead seabream (*Sparus aurata*). The potency of the tested lights to suppress melatonin levels in the plasma were ranked in the following order: white >green >blue >red. On the other hand, light suppression of melatonin levels in the pineal organ ranked: white >blue >green >red. The results demonstrate that the suppression of melatonin by light depends on the wavelength of the light and the circadian phase. In addition, male maturation in pinealectomized fish was delayed almost three weeks.

Bainard and co-authors (2001) proposed that equally wavelengths in the visible spectrum, together with UV-A wavelengths, play a role in mammalian photoperiodism in natural habitats. We applied a visible spectrum study of a representative of marine perciform, the gilthead seabream which has pineal organ that includes photodetectors, circadian clock, and a complete melatonin synthesis mechanism (Ron and Okimoto, 1999). Effects of full-spectrum white (at normal room intensities) and monochromatic light (at various wavelengths) on pineal (*in vitro*) and plasma (*in vivo*) melatonin were investigated in the gilthead seabream during the naturally dark hours of the day (18:00 to 06:00). The evaluated zenith wavelengths (λ max) of the intensity treatments tested in the *in vivo* experiment were 434 nm (blue) with a light intensity of 5 lux, 548 nm (green) with a light intensity of 20 lux, 614 nm (red) with a light intensity of 50 lux and white light with an intensity of 550 lux. The treatments for the *in vitro* experiment were 434 nm (blue) with a light intensity of 60 lux, 548 nm (green) with a light intensity of 100 lux, 614 nm (red) with a light intensity of 80 lux and white light with a light intensity of 800 lux. The potency of the tested lights to suppress melatonin levels in the plasma were ranked in the following order: white >green >blue >red. On the other hand, light suppression of melatonin levels in the pineal organ ranked: white >blue >green >red. The results demonstrate that the suppression of melatonin by light depends on the wavelength of the light and the circadian phase.

Howell and coworkers (2003) showed that photoperiod manipulation could be use to modify the time of reproduction in black sea bass and raise the quantity of eggs for aquaculture operation. We tested the consequences of applying monochromatic light in gilthead sea bream aquaculture and found that the growth rate improved with white and blue light before and during the reproductive season. There were significant differences between the gonad weights of the treated groups and those of the control groups. Another experiment tested the effect of pinealectomy on gonad maturation. The results showed that male maturation in pinealectomized fish was delayed almost three weeks compared with that of sham-operated and control animals (Figure 1 and 2). In addition, we investigated the effects of melatonin given in the food on gonad maturation. Our findings showed that the melatonin treatment affected the gonadosomatic index and that the pineal organ has an important role in the alteration of the reproductive system in fish via pineal melatonin.

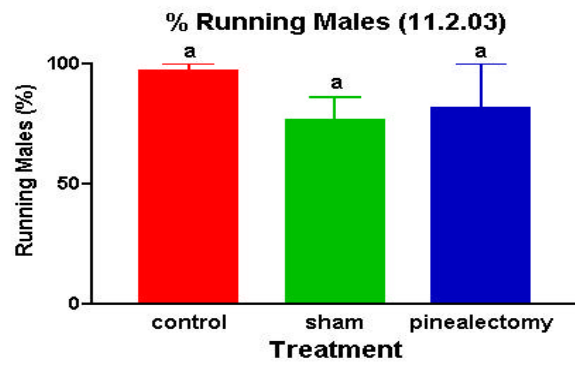
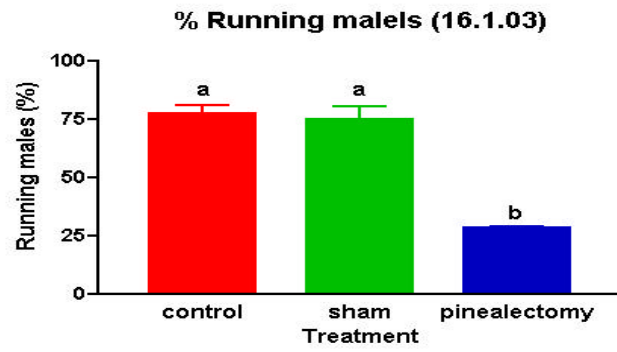


Figure 1. Percentage of running seabream males 16 vs. 42 days post pinealectomy.

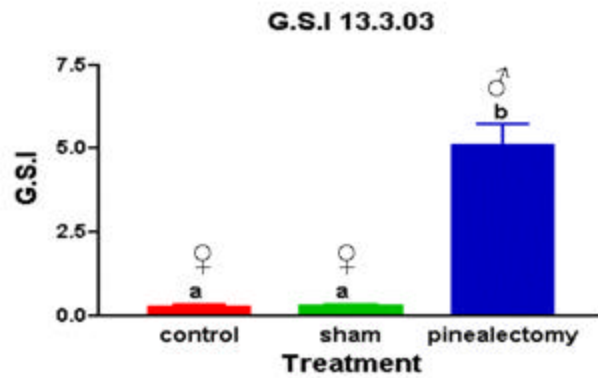


Figure 2. Gonadal somatic index of seabream 72 days post pineaectomy.

Acknowledgements

We thank Keren Bressler, Nitzan Segev, Gilad Heinisch, Adi Peduel and Mirit Gada for their assistance. This work was supported by a grant from Suf Fish Farm Ltd., Eilat, Israel to B. Ron.

References

- Brainard, G.C., Hanifin, J., Barker, F.M., Sanford, B. and Stetson, M.H. 2001. Influence of near-ultraviolet radiation on reproductive and immunological development in juvenile male Siberian hamsters. *J. Exp. Biol.* 204:2535-2541.
- Howell, R.A., Berlinsky, D.L. and Bradley, T.M. 2003. The effects of photoperiod manipulation on the reproduction of black sea bass, *Centropristis striata*. *Aquaculture* 218:651-669.

Ron, B., and Okimoto, D.K. 1999. Melatonin release from the pineals of two sparids: *Sparus aurata* and *Acanthopagrus bifasciatus*. *Adv. Exp. Med. Biol.* 460:73-77.

**MELATONIN EFFECTS ON METABOLISM
AND BEHAVIOURAL RHYTHMS IN GOLDFISH**

López-Olmeda, J.F.; Bayarri, M.J.; Rol de Lama, M.A.;
Madrid, J.A.; Sánchez-Vázquez, F.J.
Department of Physiology, Faculty of Biology, University of Murcia,
30100 Espinardo, Murcia, Spain

EXTENDED ABSTRACT ONLY: DO NOT CITE

Introduction

Melatonin, the hormone synthesized by the pineal organ and the retina, has several functions, the main being the control of circadian and seasonal rhythms. This compound has also been described to influence feeding and locomotor rhythms (Pinillos *et al.*, 2001; Zhdanova *et al.*, 2001), as well as glucose homeostasis in fish (Delahunty & Tomlinson, 1984). In addition, in recent years it has been described as a potent antioxidant (Reiter *et al.*, 2000). The aim of this research was to evaluate the effect of melatonin administration on goldfish metabolism and its ability to act as an antioxidant. Furthermore, melatonin effects on goldfish behavioural rhythms, food intake and locomotor activity, were also investigated.

Materials and Methods

The effect of melatonin, administered intraperitoneally (i.p.) (3 mg/kg), on feeding and activity rhythms was evaluated. Melatonin administration was carried out at mid light (ML), mid dark (MD), and after a one hour light pulse at MD and constant light (LL). In addition to these acute tests, chronic administration of melatonin (3 mg/kg) during ten days was also investigated. Administration of vehicle in the same conditions was also carried out to test the influence of fish manipulation.

In order to evaluate the *in vivo* capacity of melatonin as an antioxidant, goldfish were subjected to oxidative stress caused either directly by hydrogen peroxide (H₂O₂) baths or indirectly by hypoxia and subsequent reoxygenation. Mortality

and lipid peroxidation in fish subjected to oxidative stress were measured. In addition, melatonin effect on glucose metabolism after food intake was investigated: glucose levels were measured in intact fish and after an i.p. injection of melatonin.

Results and Discussion

Melatonin administration caused a decrease on total food intake and activity, whereas vehicle administration did not show any effect on locomotor activity and even increased food intake. In goldfish maintained under LL, food intake was reduced both by vehicle and melatonin, but melatonin administration caused a significantly lower food intake than vehicle. Activity of fish in LL was similar between melatonin and vehicle injected fish. After a light pulse at MD, both melatonin and vehicle showed a decrease in food intake. Surprisingly, whereas melatonin injected fish showed a decrease of activity (10%), vehicle fish showed a marked increase of locomotor activity (50%). Chronic treatment of melatonin in goldfish also caused an inhibition on total food intake. The first day of chronic treatment occurred a marked inhibition (36%), while the second day feeding inhibition was lower than the first day (18%) and was maintained around this level during the following days of chronic treatment.

These results suggest that melatonin administration has a sedative effect on goldfish inhibiting food intake, as has been previously observed (Pinillos *et al.*, 2001). However, these effects depended on the light regime used. In addition, the effect on feeding and activity seem to be related, and the decrease of food intake caused by melatonin could be explained by the decrease of locomotor activity.

After the oxidative stress treatments, results obtained showed that melatonin only decreased lipid damage in muscle after hypoxia/reoxygenation (16,0 μM lipid peroxides/g tissue vs 33,2 μM /g tissue in the control group). In addition, mortality was not attenuated by melatonin. Furthermore, its effects on glucose metabolism caused an increase of mortality when melatonin was administered before hypoxia period. Since anaerobic glycolysis is the only energy source for goldfish under anoxia, and melatonin had a hypoglycaemic effect, any beneficial melatonin antioxidant effect during hypoxia is counteracted by its interference with glucose metabolism. In conclusion, melatonin had little effect, if any, preventing lipid peroxidation and mortality caused by oxidative stress.

Acknowledgements

This study was supported by the Spanish Ministry of Science & Tecnology (MCYT) project. No. AGL2001-0593-C03-01 to FJSV.

References

- Delahunty, G.; Tomlinson, M. Hypoglycemic effects of melatonin in the goldfish, *Carassius auratus*. *Comparative Biochemistry and Physiology* **78A**:871-875; 1984.
- Pinillos, M. L.; De Pedro, N.; Alonso-Gómez, A. L.; Alonso-Bedate, M.; Delgado, M. J. Food intake inhibition by melatonin in goldfish (*Carassius auratus*). *Physiology and Behavior* **72**:629-634; 2001.
- Reiter, R. J.; Tan, D. X.; Acuña-Castroviejo, D. Melatonin: mechanisms and actions as an antioxidant. *Curr. Top. Biophys.* **24**:171-183; 2000.
- Zhdanova, I. V.; Wang, S. Y.; Leclair, O. U.; Danilova, N. P. Melatonin promotes sleep-like state in zebrafish. *Brain Research* **903**:263-268; 2001.

**ESTIMATION OF PLASMA MELATONIN FLUCTUATION
BY CANULATION
IN TROPICAL FISH**

Y. Nikaido,
Tropical Biosphere Research Center, University of the Ryukyus,
3422 Sesoko Motobu, Okinawa 905-0227, Japan;
Tel:+81-980-47-6215/Fax: +81-980-47-4919
e-mail:yoshiaki-n@h3.dion.ne.jp

A. Takemura
Tropical Biosphere Research Center, University of the Ryukyus, 3422
Sesoko Motobu, Okinawa 905-0227, Japan

EXTENDED ABSTRACT ONLY - DO NOT CITE

Introduction

Melatonin is synthesized in the pineal organ and the retina of teleost fishes, and plays important roles in a variety of physiological functions concerning daily rhythm (Bromage et al. 2001). Melatonin fluctuation with an increase in nighttime and a decrease in day-time has been well documented in many fish species. Because of limitation of sample collection, however, few studies have been conducted on individual melatonin fluctuation. The aim of present study was to evaluate plasma melatonin fluctuation of the Mozambique tilapia (*Oreochromis mossambicus*) individually by means of a cannulation technique. We assessed daily fluctuation of plasma melatonin concentration and effect of acute changes in different light intensities on the concentration. We also paid attention to plasma melatonin fluctuation after exposing the fish to moonlight conditions at the new and the full moon.

Materials and Methods

Tilapia were collected from rivers in the Okinawa Prefecture, Japan, using a casting net and maintained in outdoor tanks (1 ton) with circulating and filtrating

freshwater at Tropical Biosphere Research Center, University of the Ryukyus, Okinawa, Japan. One week before the onset of experiments, the fish were transferred to indoor tanks (200 liter) and reared at ambient water temperature under the constant light-dark condition (12L12D, light on 08:00-20:00). After light anesthetization of the fish with MS-222, the cannula was inserted into the ventral aorta. Collection of blood sample from the fish reared at 12L12D was done every 3 h (Experiment 1). At 24:00, the fish were exposed to different light intensities (1500 to 0.1 lx) (Experiment 2) and moonlight intensities at the full and the new moon (Experiment 3). The blood samples of both experiments were collected at 0, 30 and 60 min after exposing the fish to each condition. The quantity of plasma melatonin was determined by time-resolved fluoroimmunoassay (TR-FIA) according to the method of Yamada et al. (2002).

Results and Discussion

Plasma melatonin concentrations of all the individuals were high from 21:00 to 06:00 (dark period) and low from 09:00 to 18:00 (light period). There were individual variations in increases in plasma melatonin concentration during the dark period (Figure 1). These results suggest that light condition has effect on fluctuation of plasma melatonin concentration and that sensitivity to the darkness differs among individuals.

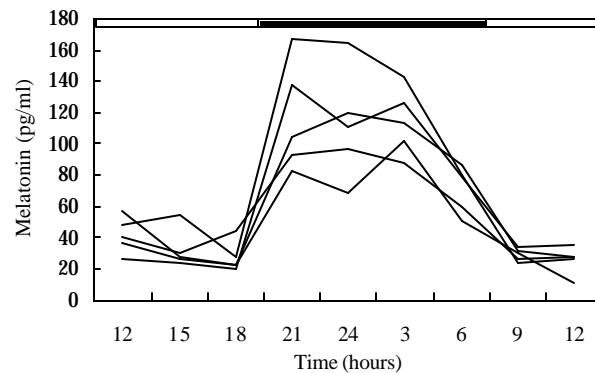


Figure 1. Individual evaluation of daily melatonin fluctuation (12L12D) in the plasma of tilapia. Upper bar in the figure shows light-dark cycle.

Exposing the fish to several light intensities (1500 to 0.1 lx) and moonlight intensities at the full and the new moon suppressed plasma melatonin concentration to basal levels significantly within 30 min. These results indicate that tilapia can perceive intensities not only of daylight but also of moonlight. We conclude that the present technique is very useful for estimation of plasma melatonin concentration under various experimental conditions.

References

- Bromage, N., Porter, M. and Randall, C. (2001) The environmental regulation of maturation in farmed finfish with special reference to the role of photoperiod and melatonin. *Aquaculture* 197: 63-98.
- Yamada, H., H. Chiba, M. Amano, M. Iigo and M. Iwata. 2002. Rainbow trout eyed-stage embryos demonstrate melatonin rhythms under light-dark conditions as measured by a newly developed time-resolved fluoroimmunoassay. *Gen. Comp. Endocrinol.* 125: 41-46.

Acknowledgements

This study was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan (AT).

**DEVELOPMENT OF THE PINEAL MELATONIN-GENERATING
SYSTEM IN GILTHEAD SEABREAM (*SPARUS AURATA*)
EMBRYOS AND LARVAE**

Benny Ron

National Center for Mariculture, Israel Oceanographic and Limnological
Research, P.O.B. 1212, Eilat 88112, Israel
P: 972-8-6361439 F: 972-8-6375761
ronbenny@agri.huji.ac.il

Galit Lisaey

National Center for Mariculture, Israel Oceanographic and Limnological
Research, P.O.B. 1212, Eilat 88112, Israel

Yoav Gothilf

Department of Zoology, George S. Wise Faculty of Sciences, Tel Aviv
University, Tel Aviv 61390, Israel

EXTENDED ABSTRACT ONLY: DO NOT CITE

Melatonin production is directly controlled by the activity of the enzyme serotonin-*N*-acetyltransferase (AANAT) that, in turn, is regulated by the circadian clock (Klein et al., 1997). The circadian clock regulation of AANAT activity differs from one species to another and could occur at the transcriptional, post-translational or both levels. In fish, the endogenous circadian clock is contained within the photoreceptive cells of the pineal gland and the retina (Ron and Okimoto, 1999). As a consequence, circadian rhythmicity of AANAT activity can be observed when these tissues are placed in culture. In this work, we investigated the very early development of seabream pineal photoreceptors and the circadian clock function by measuring melatonin, AANAT activity and sbAANAT-2 mRNA expression.

It has been shown that two AANAT genes, AANAT-1 and AANAT-2, with different expression patterns are present in the teleost species pike, trout, and zebrafish (Coon et al., 1999; Mizusawa et al., 2000). We discovered that seabream also has two AANAT genes. Our investigation revealed that

sbAANAT-1 is expressed only in the retina and sbAANAT-2 is expressed only in the pineal gland. Using Northern blot analysis AANAT1 mRNA was detected as a single 2.2 kb band only in the retina but was not detected in the pineal gland. AANAT2 mRNA was exclusively detected as a single 1.6 kb band in the pineal gland (Figure1). This differential spatial expression pattern is in accordance with the tissue distribution of the two AANATs in pike and trout.

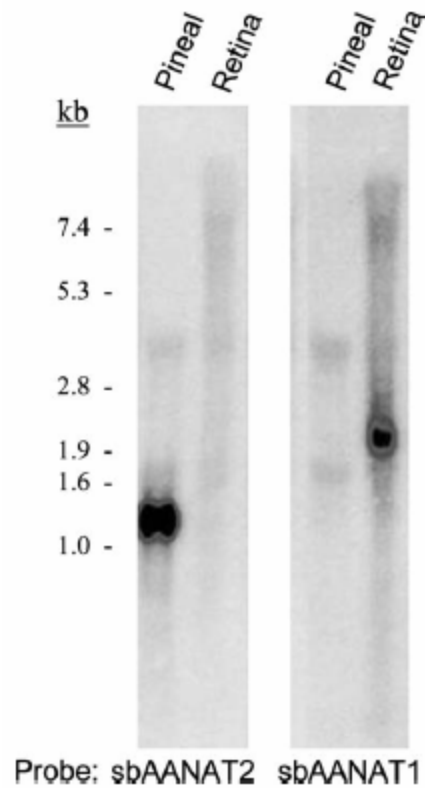


Figure 1. Northern blot analysis of seabream AANAT1 and AANAT2 mRNA expression. Total RNA was extracted from seabream retina (30 μ g) and pineal glands (5 μ g), blotted, and hybridized using radio-labeled sbAANAT1 or sbAANAT2 probes. The tissue, retina or pineal, is given above each lines and the probes are given at the bottom of the gel.

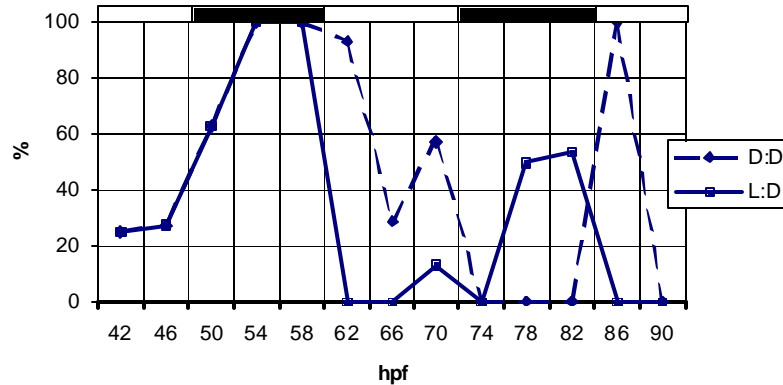


Figure 2. Seabream embryos and larvae AANAT gene expression (Y axis = % from highest value) starting at 4 h prior to hatching (n = 10) plotted over time (hours post fertilization = hpf). The white and black bars at the top of the graph represent the light and dark periods (L = 3000 lux) for the L:D treated animals. Solid black line represents the results from the L:D treated group while the dashed line represents the D:D treatment.

We then ascertained that the sbAANAT-2 mRNA is an effective marker for developmental studies of the pineal and retinal photoreceptor. The melatonin generating system is functional immediately following hatching in seabream larvae. There is a significant difference between “immediately after” (42 hpf), “before”(18 hpf) and “12 hours after” (54 hpf) hatching in the expression of the gene, enzyme activity and melatonin production. It is possible that the rhythms of sbAANAT expression are in different phases. Therefore, studying the differential regulation of these two related clock-controlled genes in seabream may provide valuable insights into the mechanism of the circadian clock.

Acknowledgements

We thank the hatchery team of NCM and Barak Yarden from ARDAG for their assistance. We also wish to thank Dr. David Klein and Dr. Steven Coon (NIH, Maryland, USA) for their useful suggestions throughout this study. This work was

supported by a grant from the Israel Science Foundation (Q32/00-17.2) to Y. Gothilf and B. Ron.

References

- Coon, S.L., Begay, V., Deurloo, D., Falcon, J., Klein, D.C. 1999. Two arylalkylamine *N*-acetyltransferase genes mediate melatonin synthesis in fish. *J. Biol. Chem.* 274:9076-9082.
- Mizusawa, K., Iigo, M., Suetake, H., Yoshiura, Y., Gen, K., Kikuchi, K., Okano, T., Fukada, Y., Aida, K. 1998. Molecular cloning and characterization of a cDNA encoding the retinal arylalkylamine *N*-acetyltransferase of the rainbow trout, *Oncorhynchus mykiss*. *Zool. Sci.* 15:345–351.
- Klein, D.C., Coon, S.L., Roseboom, P.H., Weller, J.L., Bernard, M., Gastel, J.A., Zatz, M., Iuvone, P.M., Rodriguez, I.R., Begay, V., Falcon, J., Cahill, G.M., Cassone, V.M., and Baler, R. 1997. The melatonin rhythm-generating enzyme: molecular regulation of serotonin *N*-acetyltransferase in the pineal gland. *Recent Prog. Horm. Res.* 52:307-357.
- Ron, B., and Okimoto, D.K. 1999. Melatonin release from the pineals of two sparids: *Sparus aurata* and *Acanthopagrus bifasciatus*. *Adv. Exp. Med. Biol.* 460:73-77.

**DIFFERENTIAL REGULATION
OF TWO ARYLALKYLAMINE-*N*-ACETYLTRANSFERASE
IN THE GILTHEAD SEABREAM**

Benny Ron
National Center for Mariculture, Israel Oceanographic and Limnological
Research, P.O.B. 1212, Eilat 88112, Israel
P: 972-8-6361439 F: 972-8-6375761
ronbenny@agri.huji.ac.il

Nitzan Segev
National Center for Mariculture, Israel Oceanographic and Limnological
Research, P.O.B. 1212, Eilat 88112, Israel

Bina Zilberman-Peled
Department of Zoology, George S. Wise Faculty of Sciences, Tel Aviv
University, Tel Aviv 61390, Israel

Yoav Gothilf
Department of Zoology, George S. Wise Faculty of Sciences, Tel Aviv
University, Tel Aviv 61390, Israel

EXTENDED ABSTRACT ONLY: DO NOT CITE

In-vitro experiments revealed that pineal seabream arylalkylamine-*N*-acetyltransferase (sbAANAT2) activity decreased in response to light exposure. On the other hand retinal sbAANAT2 activity increased in response to light exposure of cultured retinas. Increased activity of seabream retinal arylalkylamine-*N*-acetyltransferase (sbAANAT1) activity in response to light exposure occurred also *in vivo*. These results led us to hypothesize that sbAANAAT1 is not being controlled by the well-known proteasomal-proteolysis mechanism and might play an important role in the rapid adjustment of the retina to the appearance of light. Differences between photic-regulation of sbAANAT-2 *in-vivo* vs. *in-vitro* are suggesting an interaction between the retina and the pineal gland in fish.

In fish, individual photoreceptor cells in the pineal organ and retina contain complete melatonin rhythm generating systems. In the seabream this includes a photodetector, circadian clock, and melatonin synthesis machinery (Ron and Okimoto, 1999); some other fish, such as the trout, lack a functional clock. The melatonin rhythm is due in part to a nocturnal increase in the activity of the arylalkylamine-*N*-acetyltransferase (AANAT) that inhibited by light (Klein et al., 1997).

In preceding work done in our laboratory we have showed that light exposure at midnight decreases the abundance of sbAANAT2 protein and activity. This phenomenon is blocked by inhibitors of the proteasomal-degradation pathway (Falcon et. al, 2001). If pineal glands are maintained under light at night, treatment with these inhibitors increases AANAT2 activity and protein. Organ culture studies with the seabream also indicate that the light-induced decrease of AANAT2 activity is prevented when proteasomal proteolysis is blocked. A cAMP-dependent pathway protects AANAT2 protein from degradation. These results provided us a clue to understanding how light regulates the daily rhythm in melatonin secretion in fish photoreceptor cells and provides evidence that proteasomal proteolysis is a conserved element in the regulation of AANAT in vertebrates.

In most vertebrates, only a single AANAT gene exists. However, lately, in a work done in our laboratory, two AANATs have been identified in the gilthead seabream (*Sparus aurata*): sbAANAT1, more closely related to AANATs found in higher vertebrates, is specifically expressed in the retina; sbAANAT2 is specifically expressed in the pineal organ (Figure 1).

In this work we examined the differential activity of the two sbAANATs in the pineal and retina of gilthead seabream (*Sparus aurata*) and their regulation by the circadian and annual clock. Consequently, we characterized and compare the two sbAANATs enzymatic activity and melatonin production for over one year while rearing the fish under ambient photoperiod. In addition, we conducted short terms *in vivo* and *in vitro* experiments where either fish or pineal gland and retina were exposed to bright light during the night.

In an *in vivo*, annual experience, every two months we sampled 6 fish every 3 hours during 24 hours (Figure 2). The fish was raised under normal ambient photoperiod. We took samples of retina, pineal gland and blood. We measured five parameters: plasma melatonin, retinal and pineal melatonin level by direct

extraction of the organ, and both AANATs activities. The results indicate of a rhythmic daily melatonin level, high at night and low at daytime, with a significant change according to the different day length during the year. The AANATs activity results were not significant, and showed some peaks around dusk or dawn. Therefore, we have performed an *in vivo* and *in vitro* experiments in which we supplied 75 minutes of artificial light pulse during mid night and measurements were taken as described above.

The results indicate on an increase in retinal sbAANAT1 activity in response to light exposure (in both *in vivo* and *in vitro* experiments), while pineal sbAANAT2 activity decreased in the *in vitro* experiment, as expected. Interestingly enough sbAANAT2 activity increased in the *in vivo* experiment. The quick decrease in sbAANAT2 as a response to light exposure was explained earlier by the process of proteasomal proteolysis. These results led us to hypothesize that sbAANAT1 has an important role in the rapid adjustment of the retina to the appearance of light. On the other hand, our *in vivo* results point at a more intricate system, which might have an effect on the sbAANAT2.

Acknowledgements

We thank Keren Bressler, Alon Naor, Gilad Heinisch, Sara Levkovich, Adi Peduel and Mirit Gada for their assistance. We also wish to thank Dr. David Klein (NIH, Maryland, USA) for his gift of AANAT antibodies and for useful suggestions throughout this study. This work was supported by a grant from the Israel Science Foundation (232/00-17.2) to Y. Gothilf and B. Ron.

References

- Falcón, J., Galarneau, K.M., Weller, J.L., Ron, B., Chen, G., Coon, S.L. and Klein, D.C. 2001. Regulation of Arylalkylamine *N*-Acetyltransferase-2 (AANAT2, EC 2.3.1.87) in the Fish Pineal Organ: Evidence for a Role of Proteasomal Proteolysis. *Endocrinology* 142:1804-1813.
- Klein, D.C., Coon, S.L., Roseboom, P.H., Weller, J.L., Bernard, M., Gastel, J.A., Zatz, M., Iuvone, P.M., Rodriguez, I.R., Begay, V., Falcon, J., Cahill, G.M., Cassone, V.M., and Baler, R. 1997. The melatonin rhythm-generating enzyme: molecular regulation of serotonin *N*-acetyltransferase in the pineal gland. *Recent Prog. Horm. Res.* 52:307-357.

Ron, B., and Okimoto, D.K. 1999. Melatonin release from the pineals of two sparids: *Sparus aurata* and *Acanthopagrus bifasciatus*. *Adv. Exp. Med. Biol.* 460:73-77.

- (A) Comparison of deduced amino acid sequences of sbAANAT1 (GenBank Accession No. AY33402) and sbAANAT2 (GenBank Accession No. AY33403). Residues linked by vertical lines are identical; vertical dots indicate similarity. Putative PKA target sites are underlined. Conserved histidines which form a catalytic site are labeled with dots. The N-terminal lysine which is assumed to serve as an ubiquitination site is boxed. Conserved residues which are hypothesized to form the hydrophobic substrate binding pocket are labeled with asterisks. Residues within the substrate binding pocket that are different in sbAANAT2 are labeled '\$.'
- (B) Dendrogram showing the phylogenetic relationships of seabream AANATs to other AANATs. Peptide sequences were used to generate the dendrogram; all residues up to and including the N-terminal PKA site, and residues including and following the C-terminal PKA site (see A) were removed prior to alignment. Sequences were aligned using Clustal W and the dendrogram was generated using Phylip software. The tree was rooted with yeast (*Saccharomyces cerevisiae*) as the outgroup. GenBank accession numbers for the peptides used to generate the dendrogram: human (*Homo sapiens*; NP_001079); rat (*Rattus norvegicus*; NP_036950); chicken (*Gallus gallus*; AAB40942); Xenopus-1a1 (*Xenopus laevis*; AY316296); Xenopus-1a2 (*X. laevis*; AY316297); zebrafish-1 (*Danio rerio*; AAQ54582); zebrafish-2 (*D. rerio*; NP_571486); puffer fish-2 (*Sphoeroides nephelus*; AAL73048); pike-1 (*Esox lucius*; AAD21316); pike-2 (*E. lucius*; AAD21317); trout-1 (*Oncorhynchus mykiss*; BAA34809); trout-2 (*O. mykiss*; AAD25333); seabream-1 (*Sparus aurata*; AY33402); seabream-2 (*S. aurata*; AY33403); and yeast (*S. cerevisiae*; NP_010356). The number following each fish label identifies the form of AANAT to which that sequence belongs.

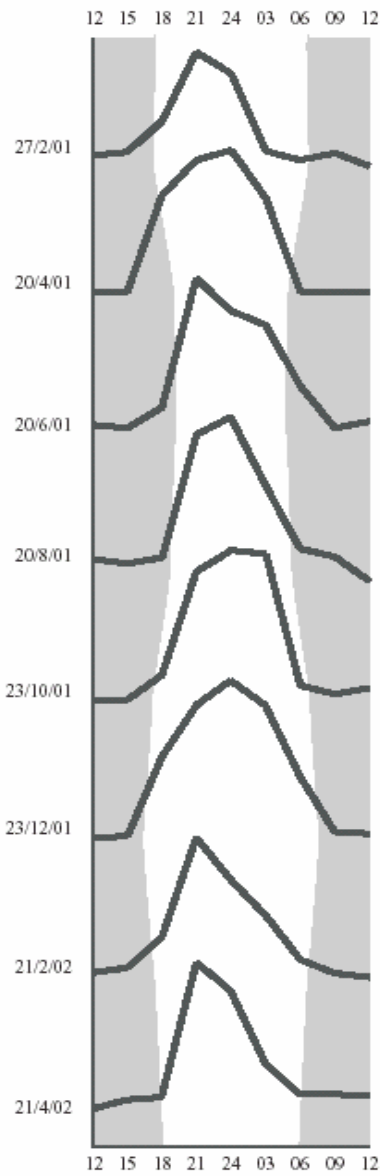


Figure 2. Plasma melatonin at 3-hour intervals in seabream (n=4 to 6) sampled at 2-month intervals for over one year. Mean values are shown for clarity without standard errors. The shaded area shows the light phase.

**CHRONOLOGY AND FOOD CONSUMPTION
OF WHITE CROAKER (MICROPOGONIAS FURNIERI),
AN ESTUARINE DEPENDENT FISH**

Gisela Mandali de Figueiredo
Laboratório de Ciências Marinhas –UNISUL,
Avenida Colombo Sales 84, Laguna CEP 88790-000, SC, Brazil,
e-mail: gmandali@hotmail.com

João Paes Vieira
Departamento de Oceanografia Biológica – Fundação Universidade Rio Grande,
Rio Grande, RS, Brazil,
e-mail: docjpv@furg.br

EXTENDED ABSTRACT ONLY – DO NOT CITE

Introduction

Feeding chronology and food consumption are influenced by many environmental factors: physical-chemical conditions, prey availability, food quality, meal size, food size, and age of fish. In the Patos Lagoon estuary (Southern Brazil) occur large fluctuations in water salinity and transparency in short period of time, which might influence the feeding behavior of fishes. The main objective of this study is to describe the feeding chronology and food consumption of white croaker (*Micropogonias furnieri*) in the estuarine environment.

Methodology

Five seasonal cruises were conducted between May 1994 and April 1995 in the Patos Lagoon estuary to collect white croaker. Samples were collected at ~ 3 hours intervals during 24 hours using bottom trawl net. Water temperature, salinity and transparency were measured.

Index of stomach fullness was calculated as the ratio of stomach contents weight and fish weight. Daily food consumption was estimated based on an exponential model using estimates of evacuation rate determined experimentally. Items from stomach contents were identified to the lowest possible taxonomic group.

Conclusion

In general, white croaker did not show a clear feeding schedule. As a result of the high turbidity of the estuarine water, it is likely that the photoperiod, an important determinant of feeding chronology, had a minor effect on the feeding behaviour of white croaker. However, when higher water transparency occurred in estuary, white croaker showed higher feeding activity before the sunset (Figure 1). This finding suggests that when white croaker is able to visualise their prey it might feed intensely during the daylight.

The daily food consumption of white croaker (90-180 mm length) ranged from 1 to 6 % of body weight d-1 and it seems to be influenced by feeding schedule. The results showed high food consumption when white croaker exhibited a feed peak. During the peak of feeding (12 to 17 h) the mean of stomach fullness were 4-fold higher than the stomach fullness in any other sampling hour. The highest ingestion during the feed peak resulted in a high food consumption compared to the majority of situations when white croaker fed continuously. Although the results suggest that the feeding schedule may cause high food consumption, further studies are still needed to approach if white croaker has a better feeding efficiency when feeding intensively in a given time.

Acknowledgements

This research was supported by CAPES and FAPERGS (Brazil), which provided Figueiredo GM with a postgraduate fellowship. We are grateful to the crew of *Larus* and the students of the laboratory of ichthyology (Biological Oceanography department - FURG) for their field and laboratory assistance.

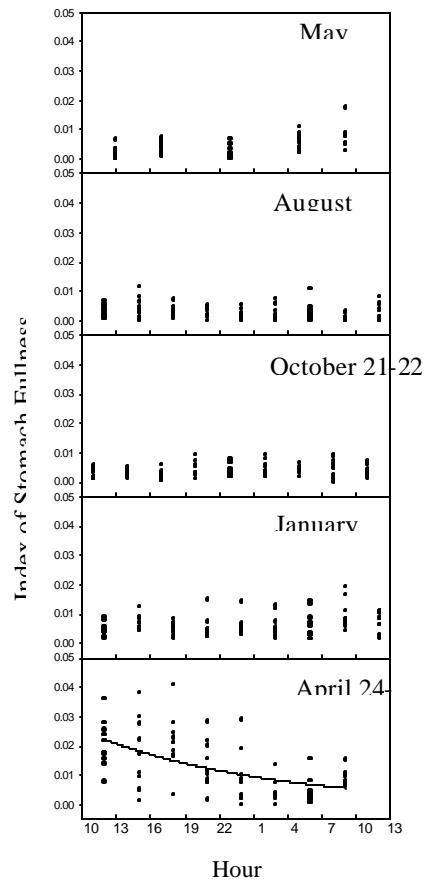


Figure 1. Index of stomach fullness (g. g-1. d-1) of *M. funieri* at each sampling trial for the 5 cruises.

**SELF-FEEDING RHYTHMS AND
MACRONUTRIENTS SELF-SELECTION
IN SENEGAL SOLE (*SOLEA SENEGALENSIS*)**

Boluda-Navarro, D.; Luz, R. K.; Rubio, V. C.; S
ánchez-Vázquez, F. J.; Madrid, J. A.
Department of Physiology. Faculty of Biology. University of Murcia. 30100
Murcia. Spain.
E-mail: dboluda@um.es

EXTENDED ABSTRACT ONLY: DO NOT CITE

Introduction

Feeding behaviour forms the basis of nutrition, one of the most important vital functions of animals. In recent years, demand-feeding systems have proved to be a useful tool to investigate the feeding behaviour of a number of species and to design new feeds adapted to the nutritional needs of fish (Sánchez-Vázquez, 1998; Aranda, 2000; Madrid, 2001; Yamamoto, 2003). Senegal sole become one of the emerging fish species with higher economical interest; however, few data are available has about its feeding behaviour and diet preferences. To date, no studies have addressed self-feeding activity in sole. Thus, the aim of this study was to elucidate whether *Solea senegalensis*, a flat fish with benthonic habits, can be trained to operate self-feeders and to test the ability of sole to feed on, and select among, three different unbalanced macronutrient diets.

Materials and Methods

To evaluate the feeding behaviour under farm conditions, we placed two feeders into each one of two raceways (4.387 soles of 594g b.w. and 7.251 soles of 375g b.w.). Each feeder had a sting sensor wich fish need bite and pull to active the feeder. All feed demands were continuously recorded and stored in a computer.

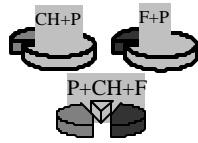


FIG 1. Experimental diets used for diet selection

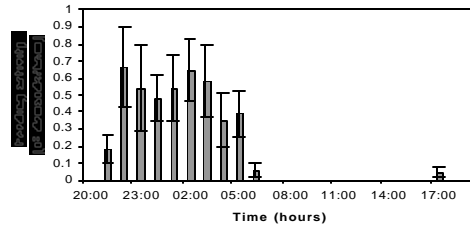


FIG 2. Demand feeding profile of fish in culture

Macronutrients selection we investigated in forty five fish of 39.7 g in b.wt. were held in 8 tanks of 75 l with constant temperature of 22.5 ± 0.5 °C and photoperiod LD 12:12. Fish were provided daily with one of three macronutrient diets. At the end of the meal the remaining pellets were counted and weighed. Animals were simultaneously fed with three diets: a protein-carbohydrate diet (PC, 75:25%), a protein-fat diet (PF, 75:25%) and a protein-fat-carbohydrate diet (PFC, 10:45:45%).

Results

Experiments of behaviour.

In culture conditions, big group of soles, learnt immediately to demand for food, so that, they spend few days to stabilize food intake. In these conditions, sole show 95% of nocturnal demands (FIG. 2).

Experiment of macronutrient selection.

When three unbalanced macronutrient diets were provided, sole selected from them to compose a complete diet, whose composition was 68% P, 15% F and 16% C.

Discussion

When soles are allowed to select the time for feeding, they prefer to do it at night. This nocturnal activity cannot be considered an artefact caused by involuntary actuation of the trigger, because nocturnal feeding activity occurred with string sensors, whose end the fish have to bite and pull to operate the feeder. These results open the possibility of using self-feeders in sole farming and clearly show the nocturnal feeding preferences of this species.

Macronutrients self-selection can be considered as a valuable methodology to design diets for Senegal sole, which preferred protein rich diet.

References

- 1.-Aranda, A., Sánchez-Vázquez, F.J., Zamora, S., Madrid, J.A., 2000. Self-design of fish diets by means of self-feeder: validation of procedures. *J. Physiol. Biochem.* 56: 155-166.
- 2.-Madrid, J.A., Boujard, T., Sánchez-Vázquez, F.J., 2001. Feeding Rhythms. In: Houlihan, D., Boujard, T., Jobling, M. (Eds.), *Food Intake in Fish*. Blackwell Science Ltd, Oxford, pp. 189-215.
- 3.-Sánchez-Vázquez, F.J., Yamamoto, T., Akiyama, T., Madrid, J.A., Tabata, M., 1999. Macronutrient self-selection through demand-feeders in rainbow trout. *Physiol. Behav.* 66: 45-51.

Acknowledgements

This research was supported by the MCYT (project AGL2001-0698 to J.A. Madrid), the EU (Concerted Action Q5CA-2001-989 to F.J. Sánchez-Vázquez). During this study D. Boluda has had a grant of the project AGL2001-0698, and R.K. Luz has had a grant of CAPES/Brazil (Coo rdenação de Aperfeiçoamento de Pessoal de Nível Superior).

